Antidepressant-like effects of Kappa Opioid Receptor Antagonists in the Forced Swim Test in Rats


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ANTI: 5'-acetamidinoethylnaltrindole
cAMP: cyclic adenosine monophosphate
CREB: cAMP response element binding protein
GNTI: 5'-guanidinonaltrindole
ICSS: intracranial self-stimulation
ICV: intracerebroventricular
U-69593: (5α,7α,8β)-N-methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4,5]dec8-yl)benzenacetamide

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ABSTRACT
We showed previously that cAMP response element binding protein (CREB) within the nucleus accumbens (NAc) of rats regulates immobility in the forced swim test (FST), an assay used to study depression. Because CREB regulates expression of dynorphin (which acts at κ opioid receptors) in NAc neurons, these findings raised the possibility that κ receptors mediate immobility behaviors in the FST. Here, we report that intracerebroventricular (ICV) administration of the κ antagonist nor-binaltorphimine (norBNI) dose-dependently decreased immobility in the FST, suggesting that it has antidepressant-like effects. Implicating a specific effect at κ receptors, similar antidepressant-like effects were seen after treatment with either of two novel, structurally-dissimilar κ antagonists: 5'-guanidinonaltrindole (GNTI), which was effective after ICV but not systemic treatment, and 5'-acetamidinoethylnaltrindole (ANTI), which was potent and effective after systemic treatment. The behavioral effects of the κ antagonists resembled those of tricyclic antidepressants (desipramine) and selective serotonin reuptake inhibitors (fluoxetine, citalopram). Conversely, systemic administration of the κ agonist U-69593 dose-dependently increased immobility in the FST, consistent with prodepressant-like effects. The effects of the κ ligands in the FST were not correlated with non-specific effects on locomotor activity. Furthermore, the most potent and effective κ antagonist (ANTI) did not affect the rewarding impact of lateral hypothalamic brain stimulation at a dose with strong antidepressant-like effects. These findings are consistent with the hypothesis that CREB-mediated induction of dynorphin in the NAc "triggers" immobility behavior in the FST. Furthermore, they raise the possibility that κ antagonists may have efficacy as antidepressants, while lacking stimulant or reward-related effects.
The neurobiology of depression is not understood. Because most antidepressants with clinical efficacy act upon monoamines (primarily norepinephrine [NE] and serotonin [5HT]), much research on depression has focused upon interactions between these neurotransmitters and their reuptake transporters and receptor proteins. However, recent research has become progressively focused upon the intracellular mechanisms of depression and antidepressant treatments (Duman, 2002; Nestler et al., 2002; Manji et al., 2001), with the goal of developing novel therapeutics that act faster, are more efficacious, and have fewer side effects. This approach has led to the study of brain circuits typically associated with reward-related processes, including the mesolimbic dopamine (DA) system (Pliakas et al., 2001; Newton et al., 2002).

The mesolimbic DA system projects from the ventral tegmental area of the midbrain to the nucleus accumbens (NAc) of the basal forebrain, and is modulated directly and indirectly by noradrenergic and serotonergic inputs (see Pasquier et al., 1977). This circuitry contributes importantly to the hedonic (rewarding) effects of food, sexual behavior, and addictive drugs (Carlezon et al., 1996b; Wise, 1998; Kreek and Koob, 1998). It has been proposed that disruption of DA function within the NAc causes anhedonia (reduced ability to experience reward) (Wise, 1982), a hallmark symptom of depression. Consistent with this notion, withdrawal from chronic amphetamine in rats causes decreases in extracellular concentrations of DA within the NAc that are accompanied by behavioral depression (Paulson et al., 1991). Similarly, cocaine withdrawal decreases glucose metabolism (Hammer et al., 1993) and reduces sodium currents within this region (Zhang et al., 1998). Chronic stress — a putative precipitator of depression — dramatically alters DA transmission within the NAc (Di Chiara et al., 1999). Together, these studies suggest that the NAc has an important role in the neurobiology of depression.

Recently, experience-dependent molecular adaptations that affect DA function within the NAc have been linked to the expression of depression-like signs in rats. Stress elevates the activity of the transcription factor CREB (cAMP response element binding protein) within the NAc shell (Pliakas et al., 2001). Elevated expression of CREB within the NAc shell increases immobility in the forced swim test (FST) (Pliakas et al., 2001), a rodent model often used to study depression (Porsolt et al., 1977). The effects of CREB
within the NAc appear related to its ability to regulate transcription of dynorphin, an endogenous κ opioid receptor ligand (Carlezon et al., 1998). Indeed, the κ antagonist norBNI (nor-binaltorphimine) attenuates the behavioral effects of elevated CREB expression within the NAc (Carlezon et al., 1998; Pliakas et al., 2001), most likely by blocking κ opioid receptors that normally inhibit neurotransmitter release from mesolimbic DA neurons (Di Chiara and Imperato, 1988; Shippenberg and Rea, 1997; Svingos et al., 1999). Recent evidence suggests that the actions of norBNI within the NAc itself are sufficient to cause an antidepressant-like effect in the learned helplessness paradigm (Newton et al., 2002). Taken together, these data raise the possibility that CREB-mediated transcription of dynorphin within the NAc decreases DA function, which triggers signs of depression.

Because of the possible connection between dynorphin and symptoms of depression, the present studies were designed to use pharmacological tools to determine if κ opioid receptors regulate depression-like signs in the FST. One advantage of the FST is that it identifies in rats treatments with antidepressant effects in humans (Porsolt et al., 1977; Detke et al., 1995). We reported previously (Pliakas et al., 2001) that high doses of norBNI, a well-characterized κ antagonist with long-lasting effects (Jones and Holtzman, 1992; Spanagel and Shippenberg, 1993), can increase the latency to become immobile in the FST, a putative indicator of antidepressant-like effects. A goal of the present studies was to extend this work by examining the effects of norBNI over a more complete range of doses, while using a more stringent and widely accepted method of scoring the FST (behavioral sampling; see Detke et al., 1995). Furthermore, to examine if the antidepressant-like effects of norBNI are associated with specific actions at κ receptors, we conducted similar studies using two novel and structurally-dissimilar κ antagonists, GNTI (5'-guanidinonaltrindole) (Jones and Portoghese, 2000; Jewett et al., 2001; Negus et al., 2002) and ANTI (5'-acetamidinoethylnaltrindole) (Stevens et al., 2000). We also explored the possibility that the κ agonist U-69593 ([5α,7α,8β]-N-methyl-N-[7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec8-yl]-benzenacetamide) would have the opposite (prodepressant-like) effects on behavior in the FST. In parallel, we examined the effects of each agent on locomotor activity. Finally, because systemic ANTI had strong
antidepressant-like effects in the FST, we examined whether it has reward-related effects using intracranial self-stimulation (ICSS) (Wise, 1998).
METHODS

Rats: A total of 591 male Sprague-Dawley rats (Charles River) were used in these studies. Rats used for forced swim testing or locomotor activity testing were housed in groups of four and weighed 325-375 gm at the time of testing, whereas those used for ICSS testing were housed singly and weighed 350-400 gm at the time of stereotaxic surgery. All rats were maintained on a 12 h light (0700-1900 h)-12 h dark cycle with free access to food and water except during testing. Experiments were conducted in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NIH) and McLean Hospital policies.

Drugs: Desipramine HCl (DMI), fluoxetine HCl (FLX), cocaine HCl (COC), norBNI, and U-69593 were obtained from Sigma (St. Louis, MO). Citalopram (CIT) was obtained from Forest Laboratories (New York, NY). GNTI and ANTI were synthesized at the University of Minnesota. Dosages of all drugs were based on their salt form. DMI, FLX, norBNI and GNTI were dissolved in distilled water, and COC and CIT were dissolved in saline. U-69593 was dissolved in 0.1 N acetic acid diluted with distilled water. All drugs were administered in a volume of 1 cc/kg, except for FLX (and the FLX-associated vehicle groups), which was administered in a volume of 2 cc/kg because of poor solubility.

Forced Swim Test (FST): Three hundred - seventy one rats were used for studies of standard psychotropic agents (DMI, FLX, CIT, COC) and kappa ligands (norBNI, GNTI, ANTI, U-69593) in the FST. The FST is a two-day procedure in which rats swim under conditions in which escape is not possible. On the first day, the rats are forced to swim for 15 min. The rats initially struggle to escape from the water, but eventually they adopt a posture of immobility in which they make only the movements necessary to keep their heads above water. When the rats are re-tested 24 hours later, immobility is increased. Treatment with standard antidepressant drugs within the 24 hr period between the first exposure to forced swimming and re-testing can block facilitated immobility, an effect correlated with antidepressant efficacy in humans (Porsolt et al. 1977; Detke et al. 1995).
No treatment was given before the first day of the FST for studies involving the standard psychotropic agents, ANTI, or U-69593. For the studies involving norBNI and GNTI, each rat was anesthetized with intraperitoneal (IP) pentobarbital (65 mg/kg), and given subcutaneous (SC) atropine sulfate (0.25 mg/kg) to reduce bronchial secretions. Intracerebroventricular (ICV) microinjections (0.3 mm posterior to bregma, 1.2 mm lateral from midsagittal suture and 4.0 mm below dura; Paxinos and Watson, 1986) of norBNI (1.25 - 20 µg) or GNTI (5.0 - 20 µg) were administered in 2.0 µl over 10 min using a Hamilton syringe with a 26-gauge needle (see Pliakas et al., 2001). At the dosages used, a single treatment with these agents has extended efficacy: norBNI produces a κ receptor-specific blockade in rats (Jones and Holtzman, 1992) for >3 weeks (Spanagel and Shippenberg, 1993). GNTI has detectable κ receptor-specific effects in monkeys for ~10 days (Negus et al., 2002), although its effects in rats do not appear to endure as long as those of norBNI (Jewett et al., 2001). (ANTI was not tested in ICV studies because it is a novel compound with modifications to its chemical structure that made it hypothetically more bioavailable by systemic injection than norBNI or GNTI). Rats recovered from surgery for two days prior to the FST.

On the first day of the FST, rats were placed in clear, 65 cm tall - 25 cm diameter cylinders filled to 48 cm with 25°C water. After 15 min of forced swimming, the rats were removed from the water, dried with towels, and placed in a warmed enclosure for 30 min. The cylinders were emptied and cleaned between rats. Rats tested with the standard psychotropic agents, ANTI, or U-69593, received 3 separate IP injections, at 1 hr, 19 hr, and 23 hr after the first exposure to forced swimming, a commonly used treatment regimen (Porsolt et al. 1977; Detke et al. 1995; Carlezon et al., 2002). Rats given norBNI, GNTI, or vehicle by ICV microinjection did not receive systemic injections. There were 7-15 rats per treatment; control (vehicle-treated) groups had the highest numbers because each daily test session included four rats, and each group of four rats contained at least one control rat.

At 24 hr after the forced swim, rats were re-tested for 5 min (300 sec) under identical swim conditions. Re-test sessions were videotaped from the side of the cylinders and scored using a behavioral sampling method (Detke et al., 1995; Carlezon et al., 2002) by raters unaware of the treatment condition. Rats were rated at 5 sec intervals.
throughout the duration of the re-test session; at each 5 sec interval, the predominant behavior was assigned to one of 4 categories: immobility, swimming, climbing, or diving. A rat was judged to be *immobile* if it was making only movements necessary to keep its head above water, *climbing* if it was making forceful thrashing movements with its forelimbs directed against the walls of the cylinder, *swimming* if it was actively making swimming movements that caused it to move within the center of the cylinder, and *diving* if it swam below the water, toward the bottom of the cylinder. (Data quantifying diving behavior is not shown in the present report because it rarely occurred, and it was not affected by any of the treatments tested.) This behavioral sampling method reportedly differentiates classes of antidepressant drugs: for example, tricyclic antidepressants decrease immobility and increase climbing without affecting swimming, whereas selective serotonin reuptake inhibitors (SSRIs) decrease immobility and increase swimming without affecting climbing (Detke et al. 1995).

Data from each agent were analyzed separately. The number of occurrences (to a maximum of 60) of each category of behavior was analyzed using separate one-way (treatment) analyses of variance (ANOVAs). Significant effects were analyzed using *post hoc* Fisher’s least significant difference (LSD) tests.

**Locomotor activity:** Two hundred-twelve rats were used to determine if the treatments examined in the FST studies alter locomotor activity. These studies were conducted exactly as the FST studies had been conducted until the point of re-testing: that is, all rats underwent the first day of the FST, and were treated with the standard agents, ANTI, or U-69593 at the normal pretreatment times (1 hr, 19 hr, and 23 hr after swimming). Rats given norBNI or GNTI received ICV microinjections two days before the first day of the FST. At 24 hr after the first exposure to forced swimming, the rats were placed for 1 hr in automated, 68 x 21 x 21 cm (L x W x H) activity chambers (Med Associates, St. Albans VT) instead of being re-tested in the FST. There were 7-12 rats per treatment; control (vehicle-treated) groups had the highest numbers because each daily test session included four rats, and each group of four rats contained at least one control rat.

The total number of activity counts (photocell beam breaks) during the test session was quantified, and data for each agent were analyzed separately. For locomotor
activity studies in which multiple doses of drug were tested (i.e., the standard psychotropic agents and U-69593), differences among treatment groups were analyzed using one-way (treatment) ANOVAs. Significant effects were analyzed further using post hoc Fisher’s LSD tests. For studies involving norBNI, GNTI and ANTI, only the highest active dose was tested, and differences from vehicle-treated rats were analyzed using Student’s t-tests.

Intracranial Self-Stimulation (ICSS): Each of 8 rats was anesthetized as described above, and implanted with a monopolar, stainless steel electrode (0.250-mm diameter; Plastics One, Roanoke, VA) aimed at the left medial forebrain bundle, at the level of the lateral hypothalamus (2.8 mm posterior to bregma, 1.7 mm lateral from midsaggital suture and 7.8 mm below dura; Paxinos and Watson, 1986). The electrodes were coated with polyamide insulation except at the flattened tip. Skull screws (one of which served as the ground) and the electrode were secured to the skull with dental acrylic.

After at least one week recovery, the rats were trained on a continuous reinforcement schedule (FR1) to respond for brain stimulation, using procedures described previously (Carlezon and Wise, 1996a). Each lever-press earned a 0.5-sec train of square-wave cathodal pulses (0.1-msec pulse duration) at a set frequency of 158 Hz. The delivery of the stimulation was accompanied by the illumination of a 2 watt house light. Responses during the 0.5-sec stimulation period did not earn additional stimulation. The stimulation current (100 - 300 µA) was adjusted gradually to the lowest value that would sustain a reliable rate of responding (at least 40 rewards per min). Once an appropriate current was found for each rat, it was held constant throughout the remainder of the experiment.

Each rat was then adapted to brief tests with each of a descending series of 15 stimulation frequencies. Each series comprised 1-min test trials at each frequency. For each frequency tested, there was an initial 5-sec "priming" phase during which non-contingent stimulation was given, followed by a 50-sec test phase during which the number of responses was counted. Following the test phase, there was a 5 sec time out period during which no stimulation was available. The stimulation frequency was then lowered by approximately 10% (0.05 log₁₀ units), and another trial was started. After
responding had been evaluated at each of the 15 frequencies, the procedure was repeated such that each rat was given 6 such series per day (90 minutes of training). During the training procedure, the range of frequencies was adjusted for each rat so that only the highest 5-6 frequencies would sustain responding. To characterize the functions relating response strength to reward magnitude, a least-squares line of best fit was plotted across the frequencies that sustained responding at 20, 30, 40, 50 and 60% of the maximum rate using customized analysis software (Steven Cabilio, Montreal, QC). ICSS threshold was defined as the frequency at which the line intersected the x-axis (theta-0; Milliaressis et al., 1986). Drug testing started when mean ICSS thresholds varied by less than 10% over 3 consecutive training sessions.

For drug testing, three rate-frequency functions (“curves”) were determined for each rat immediately prior to drug treatment. The first curve served as a warm-up period and was discarded because it tended to be unreliable. The second and third curves were averaged to obtain the baseline (threshold and maximal response rates) parameters. Each rat then received an IP injection of drug or vehicle, and four more 15-min rate-frequency curves were obtained (1 hr of testing). All rats received the same treatments in a standardized order: the first test was with vehicle, the second test was with cocaine (2.5 mg/kg, IP), the third test was with vehicle, and the fourth test was with ANTI (3.0 mg/kg, IP). Vehicle treatments were used to ensure that the rats had recovered from prior treatment with cocaine, and to minimize the possibility of conditioned drug effects. It has been established previously that repeated treatment with cocaine (or other stimulants) does not cause progressive changes in drug sensitivity in the ICSS assay (for review, see Carlezon et al., 2001).

Effects of vehicle, COC, and ANTI on thresholds and maximal response rates over the 60 min test period were evaluated using one-way ANOVAs with repeated measures. The first and second tests with vehicle did not differ, and were combined into a single group. Significant effects were analyzed further using post hoc Fisher’s LSD tests.

**Histology:** Rats that received ICV microinjections or ICSS electrodes were overdosed with pentobarbital (130 mg/kg, IP) and perfused with 4% paraformaldehyde. The fixed
brains were sliced in 40-µm sections for cresyl violet staining to confirm ICV microinjection and electrode placements.
RESULTS

Each of the standard psychotropic agents (DMI, FLX, CIT, COC) reduced immobility in the FST, but effects on the occurrences of swimming and climbing behaviors differed among drugs (Table 1). DMI (a tricyclic antidepressant) dose-dependently decreased occurrences of immobility ($F_{4,61} = 6.97, P < 0.01$), did not affect occurrences of swimming, and increased occurrences of climbing ($F_{4,61} = 6.78, P < 0.01$). In locomotor activity studies, DMI decreased activity counts at the highest doses tested ($F_{4,42} = 9.21, P < 0.01$). In contrast, FLX (an SSRI) dose-dependently decreased occurrences of immobility ($F_{3,45} = 4.58, P < 0.01$) and increased occurrences of swimming behavior ($F_{3,45} = 5.64, P < 0.01$), but did not affect occurrences of climbing. FLX also decreased locomotor activity at doses with efficacy in the FST ($F_{4,42} = 9.21, P < 0.01$). CIT (an SSRI) had effects similar to those seen with FLX: it dose-dependently decreased occurrences of immobility ($F_{3,33} = 2.94, P < 0.05$) and increased occurrences of swimming behavior ($F_{3,33} = 4.55, P < 0.01$), without affecting occurrences of climbing. CIT began to decrease locomotor activity at doses below those with efficacy in the FST ($F_{3,30} = 4.66, P < 0.01$). Like each of the SSRIs, COC dose-dependently decreased occurrences of immobility ($F_{3,36} = 7.06, P < 0.01$) and increased occurrences of swimming behavior ($F_{3,36} = 5.86, P < 0.01$), without affecting occurrences of climbing. Unlike any of the other standard antidepressant agents tested, COC did not cause even nominal changes in locomotor activity at doses with efficacy in the FST. Higher doses of COC were not tested in the FST because they caused substantial increases in locomotor activity (5.0 mg/kg: 3479.3 ± 266.7; 10 mg/kg, 4971.8 ± 532.2) in small numbers of rats ($n = 4$, each group).

The κ antagonists (Fig. 1) produced effects in the FST that were qualitatively similar to those seen with the standard psychotropic agents. ICV microinjections of norBNI dose-dependently decreased occurrences of immobility ($F_{5,49} = 4.68, P < 0.01$) and increased occurrences of swimming behavior ($F_{5,49} = 3.85, P < 0.01$) (Fig. 2a). There was also a statistically significant effect on climbing ($F_{5,49} = 2.74, P < 0.05$), but post hoc analyses revealed that this was due to differences between rats in the norBNI 1.25 μg and norBNI 10 and 20 μg groups rather than any differences from vehicle-treated rats. There was no effect of norBNI on locomotor activity at the dose with
antidepressant-like effects (20 µg) (Fig. 2b). Similarly, ICV microinjections of GNTI dose-dependently decreased occurrences of immobility ($F_{3,35} = 3.00, P < 0.05$) and increased occurrences of swimming behavior ($F_{3,35} = 3.04, P < 0.05$), but did not affect climbing behavior (Fig. 3a). GNTI had no effect on locomotor activity at the highest dose (20 µg) with antidepressant-like effects in the FST (Fig. 3b). Systemic administration of GNTI (1.0 - 10 mg/kg, IP) had no effect in small numbers of rats (~5 per group) (data not shown). However, systemic (IP) administration of ANTI had efficacy in the FST: it dose-dependently decreased occurrences of immobility ($F_{4,44} = 2.89, P < 0.05$), but unlike the other κ antagonists, it increased occurrences of climbing behavior ($F_{4,44} = 2.88, P < 0.05$) without affecting swimming behavior (Fig. 4a). Systemic administration of ANTI did not affect locomotor activity at the highest dose (3.0 mg/kg) with antidepressant-like effects in the FST (Fig. 4b).

Systemic (IP) administration of the κ agonist U-69593 (Fig. 1) had effects in the FST that were qualitatively opposite to those seen with the κ antagonists. U-69593 dose-dependently increased occurrences of immobility ($F_{5,60} = 3.97, P < 0.01$) and decreased occurrences of swimming behavior ($F_{5,60} = 4.31, P < 0.01$), without affecting climbing behavior (Fig. 5a). U-69593 significantly decreased locomotor activity ($F_{2,20} = 16.34, P < 0.01$) at the highest dose (10 mg/kg, IP) with prodepressant-like effects in the FST, but had no effect at a lower dose (3.0 mg/kg) that also affected immobility and swimming behaviors (Fig. 5b).

In the ICSS studies, the effects on thresholds depended upon treatment ($F_{2,14} = 11.8, P < 0.01$), whereas there were no significant effects on maximal response rates. Treatment with vehicle tended to cause small, non-significant increases in ICSS thresholds (Fig 6a) and decreases in maximum response rates (Fig 6b) during the 1 hr test session. There were no differences between the effects of the first (test 1) and second (test 3) injection with vehicle, so these two sessions were combined into a single mean. The lowest dose of cocaine (2.5 mg/kg) tested with antidepressant-like effects in the FST significantly decreased ICSS thresholds without significantly affecting maximal response rates. In contrast, the highest dose of ANTI (3.0 mg/kg) tested with antidepressant-like effects in the FST did not affect ICSS thresholds or maximal response rates. ICSS electrodes were located in the medial forebrain bundle at the level of the lateral
hypothalamus, and the placements were indistinguishable from those depicted previously (Carlezon and Wise, 1996a; Carlezon et al., 2001).
DISCUSSION

Standard antidepressants (DMI, FLX, CIT) reduced immobility in the FST, a putative antidepressant-like effect (Porsolt et al., 1977; Detke et al., 1995; Carlezon et al., 2002). Moreover, the behavioral sampling scoring method (Detke et al., 1995) distinguishes among classes of drugs: as expected, DMI decreased immobility and increased climbing, whereas FLX and CIT each decreased immobility and increased swimming. Together, these findings confirm the sensitivity of our methodology to agents with antidepressant efficacy in humans.

Cocaine also reduced immobility in the FST, consistent with the antidepressant and “mood-elevating” effects of the drug (Post et al., 1974). Furthermore, cocaine increased swimming behaviors without affecting climbing behaviors. This pattern suggests that the antidepressant-like effects of cocaine resemble those of SSRIs more closely than those of tricyclic antidepressants, although the possibility that it reflects a dopaminergic mechanism cannot be discounted. Cocaine is often regarded as a prototypical dopaminergic agent, but it actually has higher affinity for 5HT transporters than for DA or NE transporters (Ritz and Kuhar, 1989). Additional work with agents with improved selectivity is needed to determine if relatively specific increases in individual neurotransmitters is associated with particular behavioral patterns in the FST.

A single ICV treatment with the κ antagonist norBNI dose-dependently decreased immobility in the FST, suggesting that this agent has antidepressant-like effects. This effect is consistent with our previous observations that norBNI decreases dysphoria likely associated with acute cocaine withdrawal (Carlezon et al., 1998; Pliakas et al., 2001). Indeed, antidepressants such as DMI reportedly reduce symptoms of cocaine withdrawal in humans (Gawin et al., 1989) and rats (Markou et al., 1992). Likewise, a single ICV treatment with GNTI, a structurally-dissimilar κ antagonist, had antidepressant-like effects. GNTI was more potent than norBNI, consistent with previous reports in which the effects of these agents were compared (Jones and Portoghese, 2000; Jewett et al., 2001; Negus et al., 2002). The fact that structurally-dissimilar κ antagonists have similar actions in the FST suggests that the antidepressant-like effects of norBNI are attributable to a specific blockade of κ receptors rather than non-specific side effects. However, GNTI was not effective by systemic administration at doses up to 10 mg/kg (IP),
suggesting that the chemical structure of this agent — specifically, the high pKa of the guanidinium group — may limit its bioavailability.

We also tested ANTI, a more lipophilic κ antagonist, using the repeated systemic (IP) administration regimen. ANTI is a potent and selective κ-selective antagonist that contains an amidinium group, which is less basic than the guanidinium group of GNTI. This modification allows a greater percent of non-ionized ANTI to enter the brain. ANTI also contains three additional hydrophobic groups (two methylenes and one methyl) that increase its lipophilic character, further contributing to improved access to the brain and greater potency. Like norBNI and GNTI, ANTI reduced immobility in the FST, providing additional evidence that antidepressant-like effects may be a general attribute of κ antagonists.

Effects on swimming and climbing behaviors differed among the κ ligands. Treatment with norBNI and GNTI increased swimming behaviors, whereas ANTI increased climbing behaviors. The significance of this observation is unclear. These data suggest that alterations in the chemical structure of κ antagonists can alter their effects on monoamine function (Detke et al., 1995). However, differential effects on the swimming and climbing measures may also involve factors other than interactions among NE, 5HT, and DA (Carlezon et al., 2002). Furthermore, systemic treatment with a κ agonist had effects in the FST that were opposite to those seen with the antagonists: U-69593 increased immobility and decreased swimming. These data suggest that U-69593 has prodepressant-like effects in rats, which is consistent with previous observations that κ agonists produce dysphoria in humans (Pfeiffer et al., 1986). Considering together the effects of the κ agonist and the antagonists, these findings raise the possibility that κ opioid receptors mediate signs of immobility in the FST.

One concern when using the FST is that non-specific treatment effects on activity levels could complicate data interpretation. If treatments increase activity, they could appear to reduce immobility in the FST and thus be incorrectly identified as antidepressants. Accordingly, we conducted locomotion studies in parallel with the FST studies to identify potentially confounding effects. Locomotion studies were conducted exactly as the FST studies, except during the second day of testing the rats were placed in activity chambers rather than being re-exposed to forced swimming. If anything, the
standard antidepressants tended to decrease locomotor activity at the time of re-testing in the FST. It is unlikely that the ability of an agent to decrease locomotor activity is necessary to cause antidepressant-like actions in the FST, however, because cocaine had antidepressant-like actions without affecting activity. Furthermore, other treatments have been shown to produce antidepressant-like (or prodepressant-like) effects in the FST without affecting locomotor activity (Pliakas et al., 2001). The observation that cocaine had antidepressant-like effects in the FST without affecting locomotor activity suggests that its mood-enhancing effects occur at lower doses than its stimulant effects. Similarly, none of the κ antagonists affected locomotor activity at the highest doses with antidepressant-like effects in the FST. Although U-69593 decreased activity at the highest dose tested (10 mg/kg, IP), it did not affect activity at a lower dose (3.0 mg/kg, IP) that also had prodepressant-like effects. Because both antidepressant-like effects and prodepressant-like effects can be observed after treatments that decrease locomotion, it seems unlikely that our FST studies were affected by non-specific treatment effects on locomotor activity.

Although the mechanisms by which κ receptor agents regulate behavior in the FST are unknown, previous work suggests that altered DA function may be involved. One possibility is that immobility is mediated by κ receptors located on the terminals of mesolimbic DA neurons that project to the NAc (Svingos et al., 1999). These presynaptic κ receptors regulate DA release: administration of κ agonists directly into the NAc decreases local extracellular concentrations of DA to ~50% of baseline (DiChiara and Imperato, 1988; Maisonneuve et al., 1994). Conversely, direct administration of κ antagonists into the NAc increases DA concentrations to ~175% of baseline (Maisonneuve, 1994). These increases in concentrations of DA are modest compared to those observed after treatment with psychostimulants such as cocaine (to 400-800% baseline) or amphetamine (to > 1000% baseline) (DiChiara and Imperato, 1988; Maisonneuve et al., 1994). Modest increases in DA concentrations within the NAc may be sufficient to cause antidepressant-like effects in the FST without stimulating locomotor activity.

Another possibility is that the efficacy of κ antagonists in the FST is related to their ability to block the effects of experience-dependent elevations in endogenous
dynorphin levels. Forced swimming activates CREB within the NAc (Pliakas et al., 2001), and CREB regulates dynorphin expression within the NAc and related tissues (Cole et al., 1995; Carlezon et al., 1998). Thus forced swimming may trigger CREB-mediated increases in dynorphin transcription within the NAc, which contribute to the facilitated immobility normally observed during the second exposure to the FST (Porsolt et al., 1977; Pliakas et al., 2001). As such, κ antagonists may have antidepressant effects by blocking dynorphin-mediated reductions in extracellular concentrations of DA within the NAc, an effect associated with symptoms of depression including anhedonia (Wise, 1982; Paulson et al., 1991, Pliakas et al., 2001). Regardless of the exact mechanisms, recent evidence suggests that κ antagonist actions within the NAc itself are sufficient to cause an antidepressant-like effect (Newton et al., 2002).

If κ antagonists affect behavior through their ability to promote DA transmission in the NAc, then an obvious concern is that they may have abuse liability (DiChiara and Imperato, 1988). Drugs of abuse facilitate ICSS in rats, reducing the amounts of stimulation required to sustain responding (thresholds) (see Wise, 1998). In the present studies, cocaine significantly reduced ICSS thresholds at a dose (2.5 mg/kg, IP) with antidepressant-like effects in the FST, confirming that its “mood-elevating” effects are associated with reward-related effects that contribute to abuse liability. In contrast, ANTI did not affect ICSS thresholds at a dose with antidepressant-like effects in the FST. Thus the effects of ANTI seem similar to those of other antidepressant drugs (e.g., DMI, FLX) which, if anything, initially increase ICSS thresholds (Hall et al., 1990; Markou et al., 1992; Lee and Kornetsky, 1998). The fact that effective doses of ANTI lack stimulant effects in locomotor activity studies and rewarding effects in ICSS studies is early evidence that this agent lacks abuse liability at therapeutic concentrations. Studies examining the effects of ANTI and other κ ligands on ICSS behavior across a wide range of doses are in progress.

The present studies raise the possibility that κ antagonists may be a new approach to the treatment of depressive disorders. A limitation of norBNI and GNTI is low bioavailability after systemic administration. Structural modifications designed to reduce basicity and increase lipophilicity led to the design of ANTI (Stevens et al., 2000), which was efficacious in the FST after systemic administration. Additional modifications may
yield κ antagonists with increased potency or efficacy, and provide additional insight into the therapeutic potential of this class of agents.
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FOOTNOTES

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**FIGURE CAPTIONS**

Fig. 1 Chemical structures of the κ antagonists (norBNI, GNTI and ANTI) and the κ agonist (U-69593) used in the present studies.

Fig. 2 Effects of norBNI (ICV) on behavior. (a) Treatment with norBNI in the FST decreased occurrences of immobility and increased occurrences of swimming, without affecting climbing (Means ± SEM). Testing began 3 days after ICV injections, which is well within the known duration of norBNI effects. *P<0.05, **P<0.01 compared to vehicle, Fisher’s LSD tests, 7-15 rats per group. (b) norBNI did not affect locomotor activity (Means ± SEM) at a dose with antidepressant-like effects in the FST (Student’s t-tests, 7-12 rats per group).

Fig. 3 Effects of GNTI (ICV) on behavior. (a) Treatment with GNTI in the FST decreased occurrences of immobility and increased occurrences of swimming, without affecting climbing (Means ± SEM). Testing began 3 days after ICV injections. *P<0.05, **P<0.01 compared to vehicle, Fisher’s LSD tests, 7-15 rats per group. (b) GNTI did not affect locomotor activity (Means ± SEM) at a dose with antidepressant-like effects in the FST (Student’s t-tests, 7-12 rats per group).

Fig. 4 Effects of ANTI (IP) on behavior. (a) Treatment with ANTI in the FST decreased occurrences of immobility and increased occurrences of climbing, without affecting swimming (Means ± SEM). *P<0.05, **P<0.01 compared to vehicle, Fisher’s LSD tests, 7-10 rats per group. (b) ANTI did not affect locomotor activity (Means ± SEM) at a dose with antidepressant-like effects in the FST (Student’s t-tests, 7-12 rats per group).

Fig. 5 Effects of U-69593 (IP) on behavior. (a) Treatment with U-69593 in the FST increased occurrences of immobility and decreased occurrences of swimming, without affecting climbing (Means ± SEM). (b) The highest dose of U-69593 tested (10 mg/kg) decreased locomotor activity (Means ± SEM), but a lower dose that also had prodepressant-like effects in the FST (3.0 mg/kg) did not affect
activity. *$P<0.05$, **$P<0.01$ compared to vehicle, Fisher’s LSD tests, 7-10 rats per group.

Fig. 6 Effect of various treatments on ICSS behavior. Rats were given 4 treatments in a standardized order (vehicle during the first test, cocaine during the second test, vehicle during the third test, and ANTI during the fourth test). (a) Treatment with vehicle tended to cause small, non-significant increases in ICSS thresholds (Mean ± SEM) over the course of the 1 hr test sessions. There was no difference between the two vehicle treatments, so the data were combined into a single mean. Cocaine significantly decreased ICSS thresholds at the lowest dose tested with antidepressant-like effects in the FST, whereas ANTI had no effect at the highest dose tested with antidepressant-like effects in the FST. (b) None of the treatments affected response capabilities (maximal rates). **$P<0.01$ compared to vehicle, Fisher’s LSD tests, 8 rats per group.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, IP)</th>
<th>Immobility</th>
<th>Swimming</th>
<th>Climbing</th>
<th>Locomotor Activity</th>
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<td>desipramine (DMI)</td>
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<td>2.5</td>
<td>23.6 ± 2.6</td>
<td>13.6 ± 1.9</td>
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<td></td>
<td>5.0</td>
<td>21.3 ± 2.5*</td>
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<td>10</td>
<td>14.7 ± 2.0**</td>
<td>12.5 ± 1.8</td>
<td>32.6 ± 3.2**</td>
<td>899.4 ± 86.1**</td>
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<tr>
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<td>20</td>
<td>18.0 ± 2.0**</td>
<td>14.9 ± 1.1</td>
<td>27.1 ± 2.0**</td>
<td>930.6 ± 160.1**</td>
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<td>28.0 ± 2.9**</td>
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**TABLE 1:** Effect of psychotropic drugs in the Forced Swim Test (FST) and on horizontal locomotion in activity chambers. Separate rats were used for each behavioral assay. FST data were collected during the second exposure to forced swimming, and locomotor activity studies were conducted 24 hr after an exposure to forced swimming. All data are expressed as Mean Counts ± SEM. *P < 0.05, **P < 0.01 compared to vehicle, Fisher’s LSD tests (7-15 rats per group)
Mague et al., Fig. 1
Mague et al., Fig. 2

(a) norBNI (μg, ICV) - FST

(b) Locomotor Activity

Mean Counts

vehicle 1.25 μg 2.5 μg 5.0 μg 10 μg 20 μg

immobility swimming climbing

Mean Counts

vehicle 20 μg

0 500 1000 1500 2000 2500 3000
Mague et al., Fig. 3

(a) GNTI (µg, ICV) - FST

- Immobility
- Swimming
- Climbing

(b) Locomotor Activity

- Vehicle
- 20 µg

Mean Counts

Legend:
- * Statistically significant difference
- ** Highly significant difference
Mague et al., Fig. 5

(a) U-69593 (mg/kg, IP) - FST

(b) Locomotor Activity